Modulation of abdominal pain by probiotics

Anna Lyra, PhD
DuPont Nutrition & Health
Functional gastrointestinal (GI) wellbeing

Up to 70% suffer from functional GI symptoms - ¾ do not seek medical care

Chronic functional GI disturbances common – transient disturbances a rule

Criteria exist for a large array of functional bowel disorders (FBDs)

- Irritable bowel syndrome (IBS)
- Functional bloating
- Functional abdominal bloating
- Functional constipation
- Functional diarrhea
- Functional abdominal pain syndrome
Rome III criteria for IBS
(Longstreth et al., Gastroenterol 2006)

Recurrent abdominal pain or discomfort at least 3 days per month in the last 3 months associated with 2 or more of the following

- improvement with defecation
- onset associated with a change in frequency of stool
- onset associated with a change in form of stool
Hyperglasia = increased responsivness to normally painful stimuli

Allodynia = painful response to normally non-painful stimuli

Normal pain response

Pain intensity

Stimulus intensity

Non-noxious stimuli

Noxious stimuli

Sensitization to pain
Abdominal pain is a common symptom attributed to visceral hypersensitivity

Experimental and clinical data suggest that changes in gut flora may be a basis for the variability of abdominal symptoms observed in functional gastrointestinal disorders and may be prevented by specific probiotic administration (1-4).

Microbiome – gut – brain

- Bi-directional
- Early life development essential for balanced function
- Endocrine, immune, neural and intestinal factors
- Stressors can disturb (psychological, infectious, etc.)

(Chichlowski and Rudolph, JNM, 2015)
Levels of action of probiotics
”Live microbes that, when administered in adequate amounts, confer a health benefit on the host” (FAO/WHO 2002; Hill et al., 2014)

Recilience
Composition
Metabolites

Tight junction integrity
Epithelial cell proliferation
Immune effects
Mucosal gene expression

Systemic immunity
Host health

Rijkers et al., 2010
Key regulators of pain

• 3 receptors (GPCR) are mainly involved in the regulation of pain *:
  • Mu opioid receptor (MOR)
  • Cannabinoid receptor (CB)-1
  • Cannabinoid receptor (CB)-2

• All receptors are widely expressed in the central nervous system and in peripheric tissues, like gut epithelium*:
  - Enteric nervous system
  - Lymphocytes, macrophages, DC
  - Epithelial cells

**L. acidophilus NCFM®** can reduce gut pain – pre-clinical data

- Mechanism: modulation of pain-reducing receptor expression in the intestine

- Shows direct interaction between a probiotic and host nervous system receptors

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**Lactobacillus acidophilus** modulates intestinal pain and induces opioid and cannabinoid receptors

Christel Rousseaux1–3, Xavier Thuru1–3,10, Agathe Gelot4–6,10, Nicolas Barnich7, Christel Neut1–3, Laurent Dubuquoy1–3, Caroline Dubuquoy1–3, Emilie Merour1–3, Karen Geboes8, Mathias Chamaillard1–3, Arthur Ouwehand9, Greg Leyer9, Didier Carcano9, Jean-Frédéric Colombel1–3, Denis Ardid4–6 & Pierre Desreumaux1–3

Rousseaux et al., 2007: 
**L. acidophilus NCFM** induces expression of analgesic (‘anti-pain’) receptors in tissue culture cells
Aims of the study: In vitro experiments

To determine whether particular probiotic strains:

may induce expression of mu opioid (MOR) and cannabinoid 1 and 2 (CB1 and CB2) receptors on epithelial cells

and contribute to the modulation and restoration of normal visceral pain perception
Stimulation of human epithelial cells with probiotics and intestinal bacteria

HT-29 cells

0h 1h 2h 3h

• *Lactobacillus* (100 cfu/cell)
  
  *L. acidophilus* NCFM  
  *L. salivarius* Ls33  
  *L. paracasei* Lpc37

• *Bifidobacterium* (100 cfu/cell)
  
  *B. lactis* Bi07  
  *B. lactis* Bi04

• *Escherichia coli* (100 cfu/cell)
  
  Commensal (cEc)  
  Adherent-invasive (LF82)

*: Philippe D et al. *Gut* (Epub ahead of print)

**TNFα (10 ng/ml)**
Only *L. acidophilus* NCFM strains induced significant expression of MOR, CB1 and CB2 mRNA by epithelial cells

- *L. salivarius* (Ls33) induced MOR mRNA expression
- *L. paracasei* (Lpc37), *B. lactis* Bi07 and BL04 strains, and the two controls *E. coli* were ineffective
*L. acidophilus* NCFM strains induced expression of MOR, CB1 and CB2 protein by HT-29 epithelial cells
NCFM induced MOR, CB1 and CB2 mRNA expression in vivo in mice

Balb/c mice (n=16)

DO

NCFM (10⁹ bacteria / oral administration)

D15 (sacrifice)

• Lactobacillus count in faeces
• Evaluation of inflammation (histology, TNF-α, MPO)
• MOR, CB1, CB2 expression
NCFM induced MOR, CB1 and CB2 mRNA expression in vivo in mice

- No macroscopic, histologic inflammation in mice treated with NCFM
- No modification of MPO and TNF-α colonic concentrations in NCFM treated mice compared to untreated animals
- Induction of MOR, CB1 and CB2 expression in NCFM treated mice
NCFM strains induced MOR, CB1 and CB2 expression in epithelial cells through the NFκB pathway

What is the functional role of NCFM-induced analgesic receptors?
Evaluation of the functional role of NCFM-induced analgesic receptors in rats measured by colorectal distension

Male Sprague-Dawley rats (200g)(n=40)

DO  D7  D10  D15 (sacrifice)

NCFM (10⁹ bacteria / oral administration)

Saline instillations (bid)

Butyrate instillations (bid)

Colorectal distension after inflation of a balloon inserted intrarectally and connected to a barostat system*

NCFM administration induced modulation and restoration of visceral pain perception

- NCFM decreased visceral perception allowing a 20% increase of pain threshold
- and a 44% increase of pain threshold in rat with colonic hypersensitivity
- NCFM mediated a similar effect than 1mg/kg of morphine s/c

NCFM induces MOR, CB1, CB2 expression and mediates analgesic effect in the gut

NCFM increases pain threshold in the gut
CONCLUSION

*L. acidophilus* NCFM induces MOR, CB1, CB2 expression and mediates analgesic effect in the gut
**L. acidophilus NCFM® can reduce gut pain – human intervention**

*Lactobacillus acidophilus* NCFM affects colonic mucosal opioid receptor expression in patients with functional abdominal pain - a randomised clinical study

T. Ringel-Kulka*, J. R. Goldsmith†, J. M. Carroll†, S. P. Barros†, O. Palsson†, C. Jobin†,* & Y. Ringel†

Day -14

**Diary cards**

Run in period

Day 0

**Visit 1**

Begin intervention

Intervention

Day 21–30

**Diary cards**

Visit 2

End intervention

![Graphs showing per-patient fold expression increase in MOR and CB2 expression](image)

Ringel-Kulka et al. 2014
Reduction of post-colonoscopy pain (D’Souza et al., 2015)

Probiotic: NCFM and Bi-07 $1.25 \times 10^{10}$ CFU each

Fig. 2. Kaplan–Meier survival curve for differences in pain resolution between probiotic and placebo ($P = 0.028$).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Statistic</th>
<th>Probiotic ($n = 133$)</th>
<th>Placebo ($n = 126$)</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bloating</td>
<td>Mean</td>
<td>2.000</td>
<td>2.517</td>
<td>0.111</td>
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<tr>
<td></td>
<td>Standard deviation</td>
<td>1.996</td>
<td>3.054</td>
<td></td>
</tr>
<tr>
<td>Pain</td>
<td>Mean</td>
<td>1.993</td>
<td>2.779</td>
<td>0.032</td>
</tr>
<tr>
<td></td>
<td>Standard deviation</td>
<td>2.398</td>
<td>3.361</td>
<td></td>
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<tr>
<td>Return of normal bowel habit</td>
<td>Mean</td>
<td>3.054</td>
<td>3.422</td>
<td>0.280</td>
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<tr>
<td></td>
<td>Standard deviation</td>
<td>2.198</td>
<td>3.156</td>
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</table>
Lactose intolerance symptoms

<table>
<thead>
<tr>
<th>Reference</th>
<th>Subjects</th>
<th>Delivery format</th>
<th>Dose</th>
<th>Conclusion</th>
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<tbody>
<tr>
<td>Montes RG, et al. 1995. Effect of milks inoculated with Lactobacillus</td>
<td>20; 5-16 y</td>
<td>Milk containing probiotic</td>
<td>$10^{10}$ cfu/d</td>
<td>H$_2$ excretion not reduced but symptoms alleviated; different mechanism than in the case of regular yogurt starter cultures</td>
</tr>
<tr>
<td>acidophilus or a yogurt starter culture in lactose-maldigesting children.</td>
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</table>

20 lactose mal-digesting children (5-16 yr)

Single blinded study

Symptoms and breath H$_2$ excretion evaluated

$10^{10}$ cfu NCFM® in milk (11.6g lactose)

Compared to:
- $10^{10}$ *S. thermophilus*
- Plain milk

Combined symptom score of abdominal pain, bloating, gas, cramps, flatus, abdominal rumbling

Montes, et al. 1995

Unpublished clinical trial with NCFM - Study design

- **391 subjects included**

- Divided over three treatments:
  - placebo (MCC)
  - 1 billion NCFM/day
  - 10 billion NCFM/day

- Study design:
  - 8 week run-in
  - 12 week treatment
  - 4 week washout

- Faecal samples and questionnaires:
  - 0, 4, 12 and 16 weeks

<table>
<thead>
<tr>
<th>Study Site</th>
<th>Placebo</th>
<th>L. acidophilus (low)</th>
<th>L. acidophilus (high)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Helsinki</td>
<td>92</td>
<td>92</td>
<td>92</td>
</tr>
<tr>
<td>Turku</td>
<td>39</td>
<td>37</td>
<td>39</td>
</tr>
<tr>
<td>Total</td>
<td>131</td>
<td>129</td>
<td>131</td>
</tr>
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</table>

Run-in  Randomised treatment
**Primary Objective:**
Examine the effect of probiotic capsules on alleviating irritable bowel syndrome (IBS) symptoms

**Secondary Objectives:**
Examine the effect of probiotic capsules on adequate relief of IBS symptoms
Examine the effect of probiotic capsules on elevating the IBS-related quality of life
Examine the effect of probiotic capsules on alleviating anxiety and depression
Examine the effect of probiotic capsules on stool consistency and bowel movement frequency
Assess the response effect of probiotic capsules on fecal microbiota
Assess characteristics of the fecal microbiota in relation to health status, demographic data and responsiveness to treatment
Assess the response effect of probiotic capsules on product safety

For all IBS scores the within group differences were significant.
In predefined analyses placebo effect too high to allow significant difference between treatments.

Lyra et al., unpublished
Reduction in abdominal pain among participants with moderate to severe pain at baseline

- baseline vs week 12
- baseline pain VAS score >35
- for combined active groups visceral pain reduced significantly compared to placebo

T-test for the change from BL in IBS-SSS Abdominal pain for patients with baseline > 35

ITT population

<table>
<thead>
<tr>
<th></th>
<th>Mean difference</th>
<th>95% CI Lower</th>
<th>95% CI Upper</th>
<th>Standard error</th>
<th>t-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 12: L. acidophilus (high+low) vs. placebo</td>
<td>-9.5184</td>
<td>-18.8658</td>
<td>-0.1709</td>
<td>4.7036</td>
<td>-2.02</td>
<td>0.0460</td>
</tr>
</tbody>
</table>

Lyra et al., unpublished
To conclude

Functional bowel disorders common – Visceral pain a common symptom
- Visceral allodynia and hypergiasia may sensitize to pain

Increased gut permeability, low-level inflammation, microbial imbalance can induce sensitization to intestinal pain
- Probiotics may counteract these

*L. acidophilus* NCFM can directly influence the expression of pain-relieving receptors in the gut
- Pre-clinical study demonstrating mechanism-of-action and clinical data confirming the effect